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Nasal bots... a fascinating world!

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ABSTRACT

Larvae causing obligatory myiasis are numerous and they may affect cutaneous and subcutaneous tissues, wounds, nasopharyngeal cavities (nasal bots), internal organs and the digestive tract (bots) of domestic and wild animals and humans as well. Nasal bots belong to the Family Oestridae, Subfamily Oestrinae, which includes several important genera: Oestrus, Kirkioestrus, and Gedoelstia infecting Artiodactyla (except Cervidae) in Africa and Eurasia, Cephenemyia and Pharyngomyia infecting Cervidae, Rhinoestrus infecting horses, Cephalopina infecting camels, Pharyngobolus infecting African elephants, and Tracheomyia infecting Australian kangaroos. Nasal bots are widespread in Mediterranean and tropical areas and in affected animals they induce sneezing and nasal discharge which may become caked with dust making breathing very difficult. The aforementioned species of larvae are host-specific but sometimes the may be deposited in human eyes inducing a painful opthalmomyiasis of short duration.

The first fascinating trait of these parasites is the very efficient morphological and biological adaptations to parasitism they show either as larvae or as adults, in order to facilitate their survival and search for a suitable host. Nasal bots have reached different degrees of complexity in their life cycles. Indeed, while for some species (e.g., Oestrus ovis, Rhinoestrus usbekistanicus) larvae are injected by flies directly into nostrils and develop in the sinuses before being ejected for external pupation, some other species migrate from eyes to blood before returning to nasal cavities either through the ethmoid bone (Gedoelstia hässleri) or via lungs and bronchi (Gedoelstia cristata). Moreover, larvae are very well-adapted to their environment being able to undergo through hypobiosis either inside or outside the host, according to the climatic environmental conditions and seasonality.

The second fascinating trait of nasal bots is related to host behavioural and immune responses against the infection. Host behaviour may in fact prevent larviposition and inflammatory/immune reactions limit larval development. The main pathophysiological mechanisms involve mast cells and eosinophils which destroy the larvae in sensitized animals. The intense eosinophilic reaction has side effects both locally (i.e. on the nasal mucosa) and also generally, with possible interactions with gastrointestinal strongyles (e.g., both worm burdens and fecundity decreased in lambs infected by *O. ovis*).

Infected animals (e.g., sheep, goat, camel, and donkey) firstly suffer from fly strike, when adult flies inject first stage larvae on nostrils: sheep may try to avoid fly swarms but eventually *Rangifer tarandus* can only manage a terror-stricken look! Secondly, hosts will suffer from myiasis with typical nasal discharge and sneezing related to sinusitis. Clinical manifestations may vary: for example *O. ovis* induces severe clinical signs in sheep whilst produces

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few effects in goats! These parasites are diffused in many Mediterranean and tropical countries. Unfortunately, it is commonly believed that bacterial infections induced by nasal bots are of greater clinical importance: this view is not substantiated and the control of this condition depends on treatment with macrocyclic lactones, closantel and nitroxynil. Reinfections are common, and controlling nasal bots is not so simple.

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1. Introduction

Larvae of flies belonging to the Oestridae Family and Oestrinae Subfamily include several genera which cause obligatory myiasis in Artiodactyla and Equidae, infesting the nasopharyngeal cavities and internal organs during their migration. They are never found in dermal or subdermal tissues or wounds (e.g., Hypodermatinae and Cuterebrinae) or in the digestive tract (e.g., Gasterophilinae). Despite the high prevalence and severity of infection, many breeders and veterinary practitioners still remain unaware of the importance of these parasitic agents. There are very few books and papers dealing with the subfamily Oestrinae (see Colwell et al., 2006; Guimaraes and Papavero, 1999; Papavero, 1977) which is therefore not deeply known. During the last decade, some works on genetics, pathophysiology and immunology have been done in France, Italy, Mexico and Spain and a review was published by Hall and Wall (1995).

Extensive use of macrocyclic lactones is modifying parasitic diversity (Otranto and Colwell, 2008), but in some areas of southern Europe an unusually large number of different species of bot fly suggests a high degree of oestrid biodiversity. It seems related to the movement of domestic animals in association with migrating human populations in southern Europe over thousands of years (Otranto et al., 2006). Pape (2006) summarizes taxonomy and evolution of bot flies very well. A short overview of previous and current taxonomy is reported in Table 1.

Oestrids are host-specific and therefore only a few inappropriate hosts are infected, except for humans who may suffer from larvae being deposited into eye conjunctivas inducing a short-lasting opthalmomyiasis. Among animals, only diurnal and gregarious hosts are infected, and lar-

Table 1Taxonomy of Subfamily Oestrinae (Colwell et al., 2006): nasal bots.

Family: Oestridae Subfamily: Oestrinae: Artiodactyla (except Cervidae) and Equidae, Africa and Eurasia Genus: Cephenemyia (deer nose bots) Cervidae: Holarctic Genus: Cephalopina (camel nose bot): Camelus Genus: Gedoelstia (tuberculous nasal bot): Antilopinae, Africa, South of Sahara Genus: Kirkioestrus (hairy nasal bot): Hippotraginae (Bovidae), Africa, South Sahara Genus: Oestrus (sheep nose bot): Caprinae and Hippotraginae (Bovidae), Africa and Eurasia Genus: Pharyngobolus (African elephant throat bot) Genus: Pharyngomyia (deer and gazelle throat bot): cervidae, Genus: Rhinoestrus (horse nasal bot) Suidae, Giraffidae, few groups of Bovidae, Equus Genus: Tracheomyia (kangaroo throat bot): Macropus robustus

Australia

vae have never been found in carnivorous or insectivorous species. According to Papavero (1977), oestrids may infect only 4 orders of 18 of mammals, nine families amongst 120 and 25 genera amongst 998.

2. Morphological and biological adaptations

2.1. Morphological traits

All Oestrinae are larviparous and they eject their progeny onto the host. The young first-instar larvae (L1's) present numerous spines and hooks in order to add security and mobility when "landing" on nostrils (Guitton and Dorchies, 1993; Guitton et al., 1996, 2001). In fact, they must avoid being expelled by host sneezing and they also have to move quickly into nasal cavities. Usually, second-instar larvae (L2) are located in sinuses and the size of their hooks and spines is reduced being therefore able not to be expelled sneezing. Finally, third-instar larvae (L3) have spines and hooks useful for crawling outside in the environment where they pupate. Their weight is decreased to facilitate their elimination from nasal cavities (Cepeda-Palacios and Scholl, 2000a).

2.2. Biological traits

Adults do not have functional mouthparts and they have to minimize their spent energy loss. Their large eyes facilitate the localization of potential hosts, as well as suitable mates. They are fast flyers (calyptrates) and their colour, pilosity, granules and pits represent a protection against predators. Since they do not feed, the lifetime of adults must be short and females emerge from the puparium with fully developed eggs ready for fertilization.

2.2.1. Life cycles

All oestrids are larviparous and eject their progeny into nostrils or eyes of their host. *Cephenemyia* lays larvae on the mouth and the subsequent migration occurs by burrowing into the mucous membrane of the lips, gums, tongue and hard palate towards the root of the tongue and the soft palate. It has been suggested that *Cephalopina* has a biological life cycle similar to *Gasterophilus*.

Larval instars mainly develop in nasal cavities (conchae, sinuses) or, for some genera, pharyngeal, oesophageal and tracheal regions. Later, pupariation will take place in sandy soil or perhaps, in case of *Pharyngomyia*, even in nasal cavities!

Besides the simplest life cycle as *Oestrus ovis* or *Rhinoestrus usbekistanicus* (i.e. larvae are laid on nostrils, subsequently migrate into nasal cavities and sinuses, before L3 are expelled in the outside environment), there

are some extended life cycles within the subfamily. Unlike O. ovis, L2 and L3 of Cephenemyia, Cephalopina, and Rhinoestrus are located in pharyngeal pouches or diverticula whereas Tracheomyia macropi in the trachea.

Gedoelstia cristata have a more complex migration in gnu being the L1 are placed into the eyes and after penetrating into eye blood vessels, they migrate through the right heart, lung and bronchia to nasal cavities where they moult in L2 (Zumpt, 1965). The latter part of the life cycle is similar to O. ovis. The most complex life cycle is observed in gnu where larvae of Gedoelstia hässleri like G. cristata penetrate into the blood and go straight to the subdural cavity (Zumpt, 1965). The return takes place through the ethmoid bone foramens and, finally, the development of larvae is completed in the nasal cavities.

2.2.2. Larval nutrition

So far, oestrid larvae cannot be fully reared in vitro because of many aspects related to larval nutrition and appropriate environmental conditions that still need to be investigated. Oestrid larvae are never hematophagous. They feed with plasma proteins, antibodies passing through the nasal mucosa during the inflammatory process, mucin and collagen of the basal membrane. Although their hooks and spines injure the nasal membranes, larval nutrition is not only mechanical but mainly related to a biochemical process and huge nitrogen oxide liberation has been observed. Indeed, Tabouret et al. (2001c) have shown, in vitro, that ESP could stimulate nitric oxide production inducing plasma protein leakage. This up regulation is time and dose-dependent. The presence of proteases in the excretory/secretory products (ESP) of O. ovis larvae has been clearly identified (Tabouret et al., 2003a). These proteases appear to originate mainly from the gut and are exported on the nasal or the sinusal mucosa. They are trypsin-like serine proteases and participate in extracorporeal pre-digestion of proteins which is useful for larval nutrition. A weak proteolytic activity has been demonstrated in the salivary glands but these organs are also the main source of most of the immunogenic antigens (Innocenti et al., 1995; Tabouret et al., 2001a,b,c). The presence of seven positive enzymatic activities serine subclass proteases (acid phosphatase, naphthol-AS-BI-phosphohydrolase, esterase (C4), esterase lipase (C8), leucine arylamidase, aglucosidase and Nacetyl-b-glucosaminidase) was detected in salivary gland products of O. ovis L3 (Angulo-Valadez et al., 2007). The aforementioned proteases probably induce an extracorporal pre-digestion and liquefaction of host tissues. Analogously, ESP released in the mucosa could be able to degrade the components of the extracellular matrix and the lamina of epithelium (Dorchies et al., 2006) avoiding close contact with antibodies, limiting larval damages and probably also preventing respiratory spiracle from being blocked. ESP and salivary gland products may also stimulate the host immune system for mucous production to enhance the larval production in a feedback mechanism.

Although, there are no qualitative changes in the protease profile of ESP among the three instars, the increasing bulk of proteases released on the mucous membrane by L2 and L3 parallel the increasing larval nutrient requirements. These evidences suggest that even thought ESP production continues throughout larval development it is related to the increasing larval body size and their need to acquire reserves for the nonparasitic stages. These data are supported by the curves of larval growth (Cepeda-Palacios et al., 1999).

2.2.3. Larval adaptation to environment

Either in their parasitic (L1, L2 and L3) or outside (pupae) stages, nasal bots are exposed to several detrimental factors. Their clever response to these adverse factors is hypobiosis (i.e. phases of arrested development) which is an highly efficient adaptive response to external environmental conditions. It appears at any stage of development but mainly at L1's and pupae and sometimes L2 (in case of Cephalopina titillator in camels). It is also amazing the degree of temperature synchronization needed for the activation of O. ovis L1 and adult female. Both L1 and the gravid female are indeed inactive at 5 °C; they get minimum activation at 12-16 °C, showing the highest activity at 25-28 °C. This is important since gravid flies should only shoot active larvae which migrate quickly in the nose. For this reason, L1 larvae possess numerous tegument's thermal sensilla (Colwell and Scholl, 1995). The ecological roles of L1 inside the nasal cavities are therefore to achieve the maximal establishment (survival) and growth rates and to monitor the outside temperature and variations from inside the host to regulate the intra-host larval developmental rate. The structure of L1 larvae is designed for maximum heat exchange with the host (i.e. larval cross section at the ninth segment width/height ratio = 2.05 vs. 1.8 in L2). From in vitro observations, it seems that when the larval resultant temperature of the outside and host temperatures is above 12 °C, larval activation begins. Feeding behaviour begins and reaches the optimum at 22-39 °C (unpublished data). L2 and L3 larvae are located under a relatively steady temperature and their development is continuous.

Slowing development allows larvae to survive over-crowding of too many larvae in a limited space: the number of L3's is indeed always low (Tabouret et al., 2001a,b,c). Asynchronous development explains this type of regulation. Arrested development is also a mean for escaping immune responses and finally it may be considered as a form of adaptation to local climate. In temperate areas where the winter is too cold for pupariation, or during the hot and dry season of tropical countries, there is either an overwintering or aestivation strategy are adopted with most of the larval burden consisting of L1's. The moults to L2 and L3 take place when the weather conditions are good enough for external pupariation and adult fly activity.

It appears that development of oestrids is closely related to season and the number of annual cycles depends of climatic conditions. Therefore control measures with antiparasitic treatments must be closely adjusted to the climate in every area. Necropsy surveys show clearly that kinetics of L1's is related to important adult mating activity, whereas L3 burdens indicate future adult mating activity (Tabouret et al., 2001a).

Extended pupariation outside has been observed for O. ovis by Biggs et al. (1998) allowing adults flies to wait

for the best time for emergence, mating and larviposition. Choosing the right place for burrowing and pupariation is of high importance in this species and extreme temperatures into the soil (for example above 40 °C for long periods) being lethal to pupae or leading to low adult weight with high post-emergence mortality rates (Cepeda Palacios et al., 1998).

For all species of oestrids, mating and seeking activities occur optimal on warm days, sunny and not windy days at temperatures between 20 and 30 °C (Anderson, 2006). For *O. ovis*, these parameters have been measured by Cepeda-Palacios and Scholl (2000b).

Finally, it appears that *O. ovis* life cycle development is closely related to local climate and geographical location. Clinical manifestations will be different according to the biological state of larvae. During L1 hypobiosis period there are no clinical signs. Sinusitis appears during L2 and L3 development and adult larviposition activity induces sneezing and fly strike. Of course, control measures must considering this chronology.

3. Host defences against infection

Being mesoparasites (parasites living in host cavities), oestrids display complex host/parasite relationships. Indeed, their hosts also develop numerous but often ineffective strategies of expelling them as reviewed by Dorchies et al. (2006). Briefly, it appears that antigenic and some inflammatory products produced by larvae induce inflammatory and hypersensitive reactions. In the following are summarized the host aspecific and specific reactions which limit the parasitic burden of oestrids.

3.1. Inflammatory reaction

A very surprising difference appears between *Hypoderma* myiasis causing larvae, which induces anti-inflammatory effects during deep tissue migration (Boulard and Garrone, 1978), and *O. ovis* that produces pro-inflammatory products on host mucosa for its own nutrition (Tabouret et al., 2001a,b,c). The intensity of local changes induced by *O. ovis* in the mucosae of the upper respiratory tract (e.g., bright red mucosae with a discrete oedema) is not related to larvae number. Indeed, it has been suggested that any number of larvae above 10 is potentially harmful (Biggs et al., 1998).

The most important lesions are localized in the sinus and ethmoidal mucosa. In naive sheep, the epithelial cells of the naso-sinus cavities are cylindrical, ciliated and pseudo-stratified. After natural or artificial infection, hyperplasia and metaplasia develop and the muco-ciliairy film is abraded (Dorchies et al., 2006). Many cells are positively marked as Ki67epitopes indicated a strong cellular proliferation (Nguyen Van Khanh et al., 1998).

The ultrastructural changes present a gradation, depending on the anatomical sites, the most severe lesions occurring in the sinus. The stratified epithelium is disorganized; the intercellular spaces are enlarged, with epithelial disjunctions. Some of the cells have a rounded shape, presenting signs of cellular degeneration. These ultrastructural changes are likely the result of a combination of mechanical

damage associated with effects of secreted proteases from the larvae. It is also likely that these changes favour the diffusion of antigenic secretory/excretory products through the mucosa to come in close contact with the locally recruited immune cells (Dorchies et al., 2006).

Significant differences between recruitment of inflammatory cells (eosinophils, mast cells and globule leucocytes) in naturally infected and parasite-free sheep have been observed in numbers and distribution within the mucous membrane, interglandular chorion or sub mucosa (Nguyen Van Khanh et al., 1996). Similar observations have been made in camels infected with Cephalopina titillator (Viatteau et al., 1999) and in donkeys infected by Rhinoestrus usbekistanicus (Kaboret et al., 1997). Due to histological position of eosinophils and mast cells, it can be presumed that these cells are responsible for limiting parasite larval populations and in sustaining the hypersensitivity phenomenon at the site of tissue damage during infection. In vitro, it was observed that eosinophils could kill L1's following their degranulation (Duranton et al., 1997). For mast cells, no observation confirms this hypothesis but the degranulation may be very aggressive for larvae. Sheep Mast Cell Protease (SMCP) has been identified in mucosae of O. ovis infected lambs, confirming the activity of local mast cells (J. Huntley, unpublished data). O. ovis larval establishment rate and development appeared greater and more rapid in corticoid treated lambs compared to controls suggesting that the inflammatory reaction could influence the outcome of this myiasis (Jacquiet et al., 2005).

3.2. Hypersensitivity phenomenon

Histopathological data suggests that an immediate hypersensitivity phenomenon (Type 1) is involved in the pathogenesis of ovine oestrosis. Many B and T lymphocytes and phagocytic mononuclear cells have been observed in the mucosa of the upper respiratory tract of animals infected with O. ovis larvae compared to controls. Cell numbers increase gradually from turbinates to sinuses suggesting that T (CD3+) and B (CD20+) lymphocytes. phagocytic mononuclear cells (CD68+), eosinophils, mast cells and globule leucocytes are mainly recruited where larval molts occur and therefore where L2's and L3's are present (Tabouret et al., 2003b). This cellular profile suggests a Th2-type cytokine production by ovine T lymphocytes but clear evidence to support this hypothesis is still missing in O. ovis infection. The recruitment of these cells and the presence of many macrophages suggest that antigenic presentation to Tlymphocytes and cellular cooperation are intense. A similar reaction is observed in the skin during Lucilia cuprina infection (Bowles et al., 1988) and in gastrointestinal mucosa when resistance to trichostrongyles is developing (Meeusen and Balic, 2000).

3.3. Eosinophils and consequences

Jagannah et al. (1989) were the first to describe the presence of eosinophils in upper respiratory mucosa of *O. ovis* infected sheep. Later, several experiments demonstrated an important trans-epithelial migration of eosinophils indicating that they are attracted in the lumen of the nasal

and sinusal cavities to come into contact with the larvae. According to Yacob et al. (2004), the kinetics of blood eosinophilia in weekly O. ovis experimentally infected sheep shows a primary peak in eosinophil numbers four days after the primary experimental infection followed by a higher peak after the second infection. After that, no major change occurs irrespective of the number of reinfections. Nevertheless, the number of eosinophils was always higher than in control animals until the end of the followup. This study showed that there was a large accumulation of these cells in mucosae of the upper respiratory mucosae. It was also worth of note a significant accumulation of eosinophils in the tissues of the trachea, bronchia and lungs even though O. ovis was not present there. In fact, this "distant" eosinophilic reaction may have important consequences on other parasites living in these locations even thought they are not specifically recruited by these parasites. Indeed, since mixed parasitic infection of animals is a common phenomenon in nature, synergistic or antagonistic effects between two or more parasites in a given host may occur (Cox, 2001). For instance, the establishment of O. ovis larvae in the upper respiratory tract of sheep elicits high inflammatory cellular activity in the gastrointestinal tract affecting the development and fecundity of Haemonchus contortus infection in the abomasums (Dorchies et al., 1997; Terefe et al., 2005). Similarly, Yacob et al. (2004, 2006) indicated the existence of an antagonistic interaction between the populations of O. ovis in the nasal cavities and Trichostrongylus colubriformis burdens in the small intestine. This phenomenon was also recently reported in goats (Yacob et al., 2008).

3.4. Antibody responses

Oestrus ovis infection elicits an IgM and IgG systemic antibody response in both sheep and goats (Suarez et al., 2005; Angulo-Valadez et al., 2008, 2009). Many serological tests have been developed using a crude L2 somatic extract. Innocenti et al. (1995) demonstrated that salivary glands proteins are the most antigenic O. ovis larvae proteins compared to digestive tube contents or cuticular antigens. More precisely, a 28 kDa protein complex is the most antigenic fraction of salivary gland contents (Tabouret et al., 2001b) and was used to develop an indirect ELISA test. Sensitivity, specificity, positive and negative predictive values were improved compared to the previous tests but detection of infected animals remains difficult in winter when L1 arrest their development inside the host. Negative correlations among larval establishment and/or larval development as well as intensity of local and systemic IgG responses were found in naturally infected ewes (Angulo-Valadez et al., 2008).

4. Clinical consequences

Clinical consequences of the infection are similar for all host species (e.g., sheep, goat, dromedary, horse, springbok). The intensity of the clinical features is related to sensitivity of the host species. The best example is given by comparison of sheep and goat infections. The first is very sensitive, the second one less: usually breeders are familiar

with ovine oestrosis but rarely with goat oestrosis. Clinically, three different phases of the infection appear even though they may be sometimes mixed: fly strike, sinusitis and other consequences.

4.1. Fly strike

Fly activity affects sheep behaviour. Indeed, shepherds may observe sheep getting nervous and gathering close together, keeping their noses deep inside the fleece of the neighbors or close to the ground during displacements. If air temperature is low, flies are not active: the sheep resume grazing and are spread all over the pasture. During this period there is little or none congestion of the nasal septum and turbinates and only a small amount of mucus is observed. Goats seem less sensitive to fly strike perhaps as a result of their browsing habits (Hoste et al., 2001). However, there are goat flocks showing strong behavioural defences against fly strike, as it happens in Baja California, Mexico. Running away, searching of shadow shelter during the heat of the day, and sneezing are stereotyped behaviours in the proximity of larvipositing flies. Goat kids show this reaction from the early post-weaning period as well. Goats seem to be more well-adapted to O. ovis infection than sheep, probably because O. ovis species have co-evolved for a longer time with goats rather than with sheep. It has been reported that oestrosis prevalence and larval burden is lower in goats than in sheep (Papadopoulos et al., 2006). Goats seem avoiding more effectively the larvipositing flies using these evasive behaviours: it has been proposed that goats are better adapted to O. ovis infection than sheep, probably because both O. ovis and goats have co-evolved for longer than sheep (I. Jourdane, personal communication).

On the other hand, *Cephenemyia* fly strike induces a terror-stricken look in *Rangifer tarandus*.

4.2. Rhinitis and sinusitis

A few weeks after larviposition, nasal discharge and sneezing become more evident and frequent. Sheep are agitated and the nasal discharge which is initially serous, secondly gets sero-mucous, muco-purulent and eventually, in the most severe cases, purulent, being occasionally tinged with blood. The amount of nasal discharge is not related to the number of larvae but appears to be related to the individual susceptibility and also to interactions with bacteria (Dorchies et al., 1998). In hot and dry climates, the nasal discharge caused by oestrosis gets worse making breathing very difficult. As a consequence, affected sheep and goats breathe through the mouth interfering with grazing and rumination. Subsequently this local naso-sinusal infection induces signs of generalized disease, including emaciation, which may sometimes result in death. In addition, these symptoms may affect olfaction in rams thereby reducing the effectiveness of Oestrus detection which will lead to reduced conception rates (Watson and Radford, 1960). Heavily infested sheep may exhibit neurological symptoms: including ataxia, vertigo, nystagmus and amaurosis as well as epistaxis.

As the period of fly activity continues, sheep are repeatedly infested and so antibody levels increase. Because clinical signs and annoyance of sheep are at maximum intensity, breeders usually treat their flock at this time.

Local changes in the mucosae of the upper respiratory tract are independent of the number of larvae present. These changes are characterized by bright red mucosae with a discrete edema, but their intensity cannot be correlated to the numbers of parasites. Large amounts of pus and abscesses are commonly found in the sinuses.

4.3. Pulmonary and other consequences

In some breeds of sheep, neoplastic tumors (adenocarcinoma of the pituitary mucosae, 4 to 5 cm wide) might be found. The prevalence of these neoplastic processes is close to 5% in the Lacaune ewes, the local breed from Roquefort area (Bergeaud et al., 1994).

In many cases, oestrosis is worsened by complications associated with infectious bronchopneumonia and pleuropneumonia or pasteurellosis with hyperthermia and coughing. Lung abscesses are also frequent. It may be assumed that they are related to pyogenic focus in the nasosinusal area. In some cases interstitial pneumonia is the most common lesion with interstitial emphysema, pleural adhesions and atelectasis (Dorchies et al., 1993). These atypical lesions associated with oestrosis may be linked to the visna maedi virus for which pathological expression is dependant on a continuous and nonspecific antigenic stimulation of the host as previously described by Dawson (1987).

Similar lesions have been observed in donkeys infected by larvae of *Rhinoestrus usbekistanicus* (Kaboret et al., 1997). The pathology is probably caused by a permanent antigenic stimulation during infection. In both myiases, considerable numbers of eosinophils and mast cells are accumulated in the lung parenchyma mainly in the peri-bronchial region. In the absence of any other lung parasite and any other cause of allergic pneumonia, it may be presumed that aspirated larval antigens induce the pulmonary sensitization.

5. Human infection

Adult flies may eject larvae into human eyes inducing an external opthalmomyiasis or into nasal cavities or mouths. These later cases are very rare. Human infection is relatively frequent in many countries; in Libya, the annual incidence is 10/100,000. Fortunately treatment by manual extraction of larvae is effective: diagnosis may sometimes be slow as observed in American soldiers during the 1st Gulf war and in tourists coming from the Mediterranean Basin to Northern countries where nasal bots are not endemic. Currently, most papers dealing with O. ovis report cases of human infection. If O. ovis is the most frequently involved, in some areas Rhinoestrus purpureus (and perhaps R. usbekistanicus) larvae may also be deposited on conjunctiva. There is scant information about other oestrids: however, cases of infection by Cephenemyia ulrichi have been observed in Finland (Mikkola et al., 1982). A key for differentiation of L1's of O. ovis and R. purpureus has been done by Larrousse (1921).

6. Conclusion

Is this fascinating parasite like *O. ovis* blurring the authors' vision or not? This species and, at large, the whole oestrid group are among the most surprising parasites. As a matter of fact, very "smart" morphological and biological adaptations allow the survival and extension of this oestrid under very unfavourable conditions, either outside (cold, dryness) or inside the animals facing inflammatory and immune reactions. However, despite its high prevalence and the severity of the infection, many breeders and veterinary practitioners still remain unaware of the importance of diseases induced by oestrid flies.

Conflict of interest statement

The authors declare that they have no conflict of interest with the contents of this paper in any respect.

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References

Angulo-Valadez, C.E., Cepeda-Palacios, R., Ascencio, F., Jacquiet, P., Dorchies, P., Romero, M.J., Khelifa, R.M., 2007. Proteolytic activity in salivary gland products of sheep bot fly (*Oestrus ovis*) larvae. Vet. Parasitol. 149, 117.

Angulo-Valadez, C.E., Scala, A., Grisez, C., Prevot, F., Bergeaud, J.P., Carta, A., Cepeda-Palacios, R., Ascencio, F., Terefe, G., Dorchies, Ph., Jacquiet, Ph., 2008. Specific IgG antibody responses in *Oestrus ovis L.* (Diptera: Oestridae) infected sheep: associations with intensity of infection and larval development. Vet. Parasitol. 155, 257–263.

Angulo-Valadez, C.E., Cepeda-Palacios, R., Ascencio, F., Jacquiet, Ph., Dorchies, Ph., Ramirez-Orduna, J.M., Lopez, M.A., 2009. IgG antibody response against salivary gland antigens from *Oestrus ovis* (Diptera: Oestridae) in experimentally and naturally infected goats. Vet. Parasitol. 161, 356–359.

Anderson, J.R., 2006. Adult biology. In: Colwell, D.D., Hall, M.J.R., Scholl, P.J. (Eds.), The Oestrid Flies. CABI Publishing, p. 359.

- Bergeaud, J.P., Duranton, C., Dorchies, Ph., 1994. L'œstrose ovine en Aveyron: Résultat d'une enquête sur 1036 têtes à l'abattoir de Rodez. Revue de Médecine Vétérinaire 145 (11), 863–866.
- Biggs, H.C., Mcclain, E., Muller, G.L., Anthonissen, M., Hare, K.M., 1998.
 A prediction model for strike in the sheep nasal fly, *Oestrus ovis*, in Namibia. Prev. Vet. Med. 33, 267–282.
- Boulard, C., Garrone, R., 1978. Characterization of a collagenolytic enzyme from larvae of *Hypoderma lineatum* (Insecta: Diptera: Diptera, Oestriform). Comp. Biochem. Physiol. 59B, 251–255.
- Bowles, V., Carnegie, P., Sandeman, R., 1988. Characterization of proteolytic and collagenolytic enzymes from the larvae of *Lucilia cuprina*, the sheep blowfly. Aus. J. Biol. Res. 41, 269–278.
- Cepeda Palacios, R., Jiménez, M.L., Armenta, J.A., 1998. Viabilidad del gusano del cuerno Oestrus ovis I. (Diptera: Oestridae)". durante los períodos de prepupa y pupa. In: Mem. Del XXXIII Congr. Nac. De Entomol., Soc. Mex. De Entomol. Acapulco, Gro, pp. 516–520.
- Cepeda-Palacios, R., Avila, A., Ramirez-Orduna, R., Dorchies, Ph., 1999. Estimation of the growth patterns of *Oestrus ovis* L. larvae hosted by goats in Baja California Sur, Mexico. Vet. Parasitol. 86, 119–126.
- Cepeda-Palacios, R., Scholl, P.J., 2000a. Intra-puparial development in *Oestrus ovis* (Diptera: Oestridae). J. Med. Entomol. 37, 239–245.
- Cepeda-Palacios, R., Scholl, P.J., 2000b. Factors affecting the larvipositional activity of *Oestrus ovis* gravid females (Diptera: Oestridae). Vet. Parasitol. 91, 93–95.
- Colwell, D.D., Scholl, P.J., 1995. Cuticular sensilla on newly hatched larvae of *Gasterophilus intestinalis* and *Oestrus ovis*. Med. Vet. Entomol. 9, 85–93.
- Colwell, D.D., Hall, M.J.R., Scholl, P.J., 2006. The Oestrid Flies. CABI Publishing, 359pp.
- Cox, F.E.G., 2001. Concomitant infections, parasites and immune responses. Parasitology 122, 823–838.
- Dawson, M., 1987. Pathogenesis of maedi-visna. Vet. Rec. 120, 451–454.Dorchies, Ph., Yilma, J.M., Savey, J., 1993. Lung involvement in ovine Oestrosis: prevalence of lung abscesses and interstitial pneumonia. Vet. Rec. 133, 325.
- Dorchies, Ph., Bergeaud, J.P., Van Khanh, N., Morand, S., 1997. Reduced egg counts in mixed infections with *Oestrus ovis* and *Haemonchus contortus*: influence of eosinophils? Parasitol. Res. 83, 727–730.
- Dorchies, Ph., Jacquiet, Ph., Duranton, C., 1998. Pathophysiology of Oestrus ovis infection in sheep and goats: review. Veterinary Record 142, 487–489.
- Dorchies, P., Tabouret, G., Hoste, H., Jacquiet, P., 2006. Oestrinae host-parasite interactions. In: Colwell, D.D., Hall, M.J.R., Scholl, P.J. (Eds.), The Oestrid Flies. CABI Publishing, p. 359.
- Duranton, C., Dorchies, Ph., 1997. *In vitro* culture of *Oestrus ovis* (Linné 1761) first instar larvae: its application to antiparasitic drug screening. Int. J. Parasitol. 27, 125–128.
- Guimaraes, J.H., Papavero, N., 1999. Myiasis in Man and Animals in the Neotropical Region: Bibliographic Database. Editoria Pleiade, Sao Paulo, Brazil, 308pp.
- Guitton, C., Dorchies, Ph., 1993. Etude des larves d'Œstrus ovis (Linné 1761) en microscopie électronique à balayage. Rev. Méd. Vét. 144, 687–692.
- Guitton, C., Morand, S., Dorchies, Ph., 1996. Scanning electron microscopy of larval instars and imago of *Rhinoestrus usbekistanicus* Gan 1947 (Oestridæ). Parasite 3, 155–159.
- Guitton, Ch., Perez, J.M., Dorchies, Ph., 2001. Scanning electron microscopy of larval instars and imago of *Œstrus caucasicus* (Grunin, 1948) (Diptera: Oestridae). Parasite 8, 155–160.
- Hall, M., Wall, R., 1995. Myiasis of humans and domestic animals. Adv. Parasitol. 35, 257–334.
- Hoste, H., Leveque, H., Dorchies, Ph., 2001. Comparison of nematode infections of gastro-intestinal tract in Angora and dairy goats in a rangeland environment: relations with the feeding behaviour. Vet. Parasitol. 101, 127–135.
- Innocenti, L., Masetti, M., Macchioni, G., Giorgi, F., 1995. Larval salivary gland proteins of the sheep nasal bot fly (*Oestrus ovis L.*), are major immunogens in infested sheep. Vet. Parasitol. 60, 273–282.
- Jacquiet, P., Trinh Tran, T.N., Nouvel, X., Prevot, F., Grisez, C., Hailu, T.Y., Bergeaud, J.P., Hoste, H., Dorchies, Ph., Tabouret, G., 2005. Regulation of *Oestrus ovis* (Diptera: Oestridae) populations in previously exposed and naïve sheep. Vet. Immunol. Immunopathol. 105, 95–103.
- Jagannah, M.S., Cozab, N., Vijayasarathi, S.K., 1989. Histopathological changes in the nasal passage of sheep and goats infested with *Oestrus ovis* (Diptera: Œstridés). Indian J. Anim. Sci. 59, 87–91.
- Kaboret, Y., Deconinck, P., Panguy, L.J., Akakpo, J., Dorchies, Ph., 1997. Lésions de la rhinoestrose spontanée à *Rhinoestrus usbekistanicus* (Gan 1947) chez l'âne (*Equus asinus*) au Sénégal. Rev. de Méd. Vét. 148, 123–126.
- Larrousse, F., 1921. La myiase oculaire à *Oestrus ovis* L. dans la region parisienne. Bull. Soc. Path. Exo. 11, 595–601.

- Meeusen, E.N., Balic, A., 2000. Do eosinophils have a role in the killing of helminth parasites? Parasitol. Today 16, 95–101.
- Mikkola, K., Silvennoienen, J., Hackman, W., 1982. Ophthalmomyiasis caused by the elk throat fly in man. Duodecim 98, 1022–1025.
- Nguyen Van Khanh, N.V., Bourge, N., Concordet, D., Dorchies, Ph., 1996. Recherche des mastocytes et des éosinophiles de la muqueuse respiratoire chez le mouton infesté naturellement par Oestrus ovis (Linné 1761). Parasite 3, 217–221.
- Nguyen Van Khanh, N.V., Delverdier, M., Jacquiet, Ph., Dorchies, Ph., 1998. Expression tissulaire de l'épitope Ki-67 dans le compartiment épithélial de la muqueuse nasale du mouton et de la chèvre infestés naturellement par *Oestrus ovis* (Linné 1761). Rev. Méd. Vét. 149 (12), 1109–1113.
- Papavero, N., 1977. The World Oestridae (Diptera), Mammals and Continental Drift. Series Entomologica, vol. 14. Dr. W. Junk Publisher, The Hague, 240pp.
- Papadopoulos, E., Prevot, F., Diakou, A., Dorchies, Ph., 2006. Comparation of infection rates of Oestrus ovis between sheep and goats kept in mixed flocks. Vet. Parasitol. 138, 382–383.
- Otranto, D., Stevens, J.R., Brianti, E., Dorchies, Ph., 2006. Human and livestock migrations: a history of bot fly biodiversity in the Mediterranean region. Trends Parasitol. 22. 209–213.
- Otranto, D., Colwell, D.D., 2008. Biodiversity and extinction versus control of oestrid causing myiasis in the Mediterranean area. Parasite 15, 257–260.
- Pape, T., 2006. Phylogeny and evolution of Bot Flies. In: Colwell, D.D., Hall, M.J.R., Scholl, P.J. (Eds.), The Oestrid Flies. CABI Publishing, p. 359.
- Suarez, J.L., Scala, A., Romero, J.A., Paz-Silva, A., Pedreira, J., Arias, M., Diaz, P., Morrondo, P., Diez-Banos, P., Sanchez-Andrade, R., 2005. Analysis of the humoral immune response to *Oestrus ovis* in ovine. Vet. Parasitol. 134. 153–158.
- Tabouret, G., Jacquiet, Ph., Scholl, Ph., Dorchies, Ph., 2001a. *Oestrus ovis* in sheep: improvement in control through knowledge of relative third-instar populations. Vet. Res. 32, 525–531.
- Tabouret, G., Prevot, F., Bergeaud, J.P., Dorchies, P., Jacquiet, Ph., 2001b. *Oestrus ovis* (Diptera: Oestridae): sheep humoral immune response to purified excreted/secreted salivary gland 28 kDa antigen complex from second and third instar larvae. Vet. Parasitol. 101, 53–66.
- Tabouret, G., Vouldoukis, I., Duranton, C., Prevot, F., Bergeaud, J.P., Dorchies, Ph., Mazier, D., Jacquiet, Ph., 2001c. *Oestrus ovis* (Diptera: Oestridae): effects of larval excretory/secretory products on nitric oxide production by murine RAW 264.7 macrophages. Parasite Immunol. 23, 111–119.
- Tabouret, G., Bret-Bennis, L., Dorchies, P., Jacquiet, P., 2003a. Serine protease activity in excretory–secretory products of *Oestrus ovis* (Diptera: Oestridae) larvae. Vet. Parasitol. 114, 305–314.
- Tabouret, G., Lacroux, C., Andreoletti, O., Bergeaud, J.P., Hailu-Tolosa, Y., Hoste, H., Prevot, F., Grisez, C., Dorchies, Ph., Jacquiet, Ph., 2003b. Cellular and humoral local immune responses in sheep experimentally infected with *Oestrus ovis* (Diptera: Oestridae). Vet. Res. 34, 231–241.
- Terefe, G., Yacob, H.T., Grisez, C., Prevot, F.E., Dumas, E., Bergeaud, J.P., Dorchies, Ph., Hoste, H., Jacquiet, Ph., 2005. *Haemonchus contortus* egg excretion and female length reduction in sheep previously infected with *Oestrus ovis* larvae. Vet. Parasitol. 128, 271–283.
- Viatteau, E., Nguyen Van Khanh, N.V., Jacquiet, Ph., Dorchies, Ph., 1999. Mastocytes et éosinophiles des muqueuses rhino-pharyngiennes du dromadaire infesté par *Cephalopina titillator* (Clark 1816). Rev. de Méd. Vét. 150, 353–356.
- Watson, R.H., Radford, H.M., 1960. The influence of rams on the onset of *Oestrus* in Merino ewes in the spring. Aus. J. Agric. Res. 11, 65–71.
- Yacob, H.T., Jacquiet, Ph., Prevot, F., Bergeaud, J.P., Bleuart, C., Dorchies, Ph., Hoste, H., 2004. Examination of the migration of first instar larvae of the parasite *Oestrus ovis* (Linné 1761) [Diptera: Oestridae] in the upper respiratory tract of artificially infected lambs and daily measurements of the kinetics of blood eosinophilia and mucosal inflammatory response associated with repeated infection. Vet. Parasitol. 126, 339–347.
- Yacob, H.T., Basazinew, B.K., Basu, A.K., 2008. Experimental concurrent infection of Afar breed goats with *Oestrus ovis* (L1) and *Haemonchus* contortus (L3): Interaction between parasite populations, changes in parasitological and basic haematological parameters. Exp. Parasitol. 120, 180–184.
- Yacob, H.T., Terefe, G., Jacquiet, Ph., Hoste, H., Grisez, C., Prévot, F., Bergeaud, J.P., Dorchies, Ph., 2006. Experimental concurrent infection of sheep with Oestrus ovis and Trichostrongylus colubriformis: effects of antiparasitic treatments on interactions between parasite populations and blood eosinophilic responses. Vet. Parasitol. 137, 184–188.
- Zumpt, F., 1965. Myiasis in Man and Animals in the Old World. Butterworths, London.